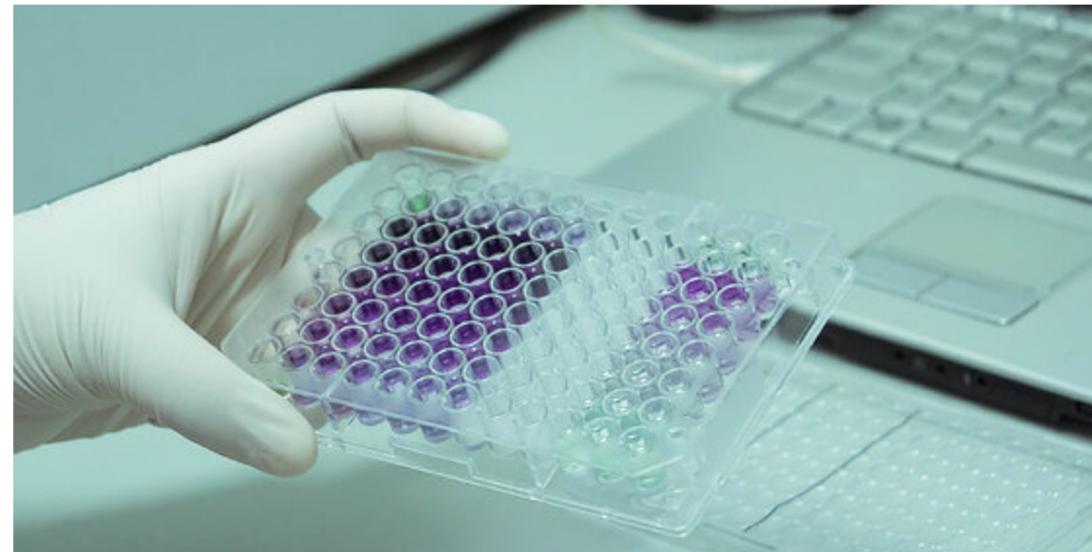
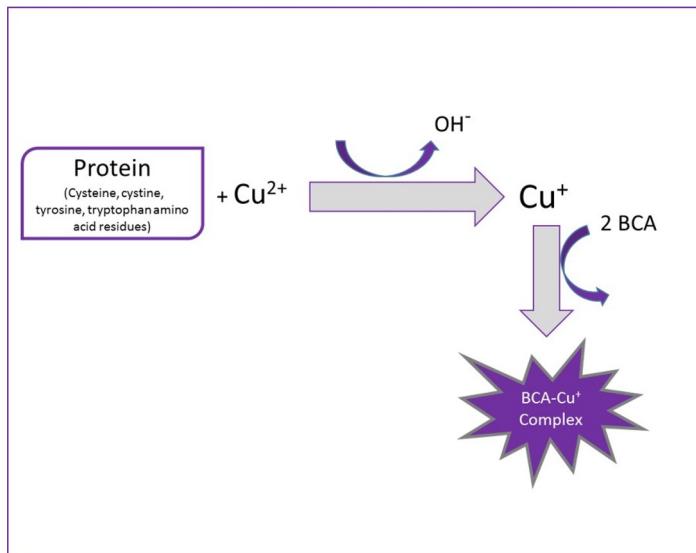


Laboratory 8

- Protein quantification of cell lysates with BCA assay
- Affinity purification of His-tagged AMY2

BCA Protein Assay



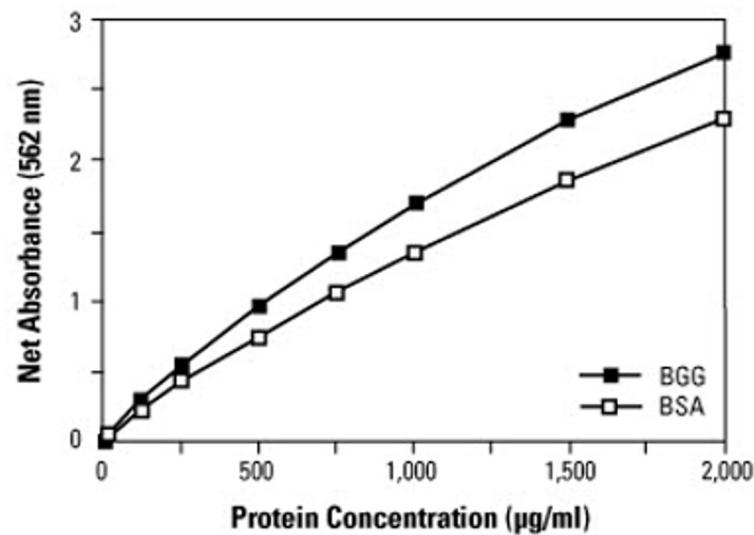
BCA Protein Assay is based on **bicinchoninic acid** (BCA) for the colorimetric detection and quantitation of total protein.

<https://www.thermofisher.com/order/catalog/product/23225#/23225>

Experimental steps

- Create a standard curve using BSA standard
- Determine the protein concentration of HEK293T whole cell lysates
 - L+ : pAmy-His and pGFP transfected cells
 - L- : Mock transfected cells
- Measure absorbance at 560 nm using a microplate reader

Example Standard Curve



Standard curves
Typical standard curves for bovine serum albumin (BSA) and bovine gamma globulin (BGG) in the BCA Protein Assay.

Source: <https://www.thermofisher.com/order/catalog/product/23225#/23225>

Affinity Purification AMY2-His fusion protein

AMY2-Myc-His His His His His His-STOP

6xHis-tag

Purification of His-tagged proteins using Ni-NTA spin column

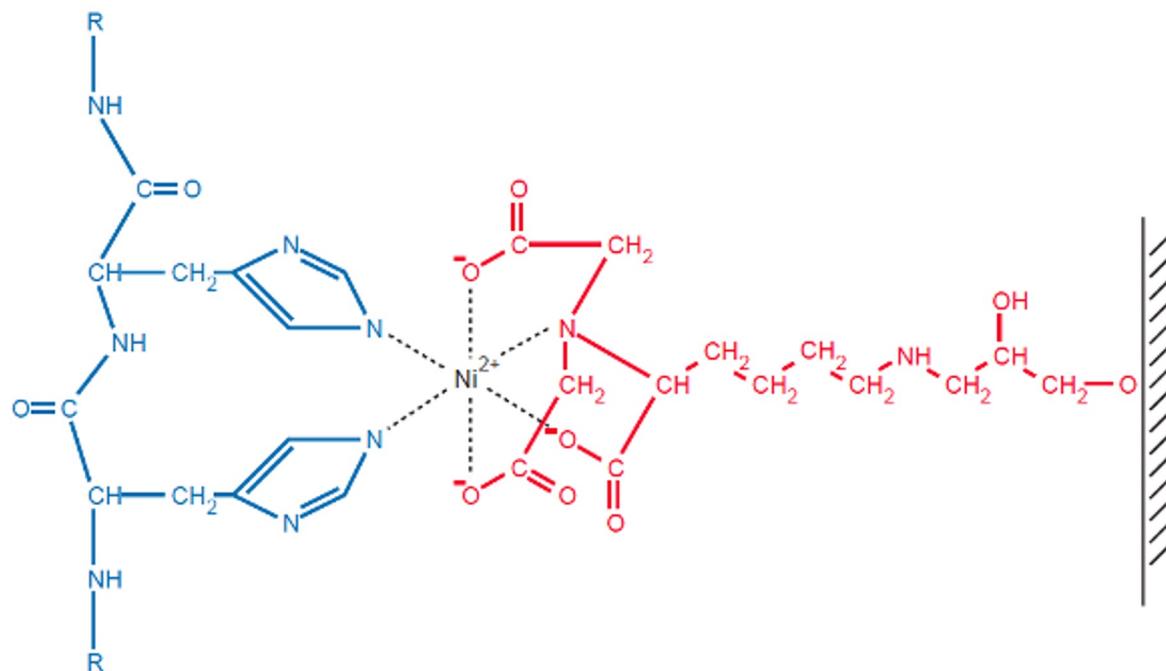
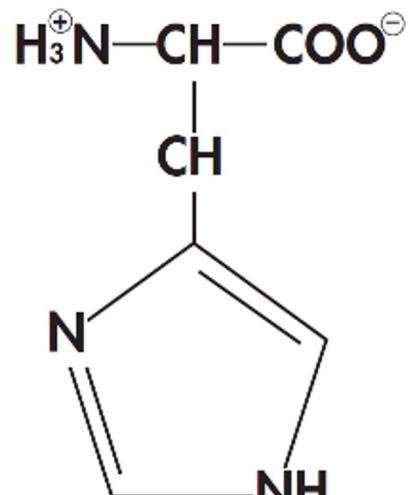


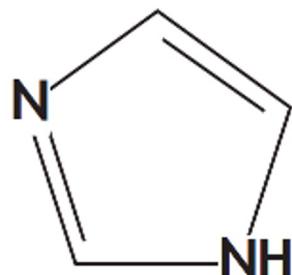
Figure 5. Interaction between neighboring residues in the 6xHis tag and Ni-NTA matrix.

Source: The QIAexpressionist Handbook,
Qiagen

Elution with Imidazole



Histidine

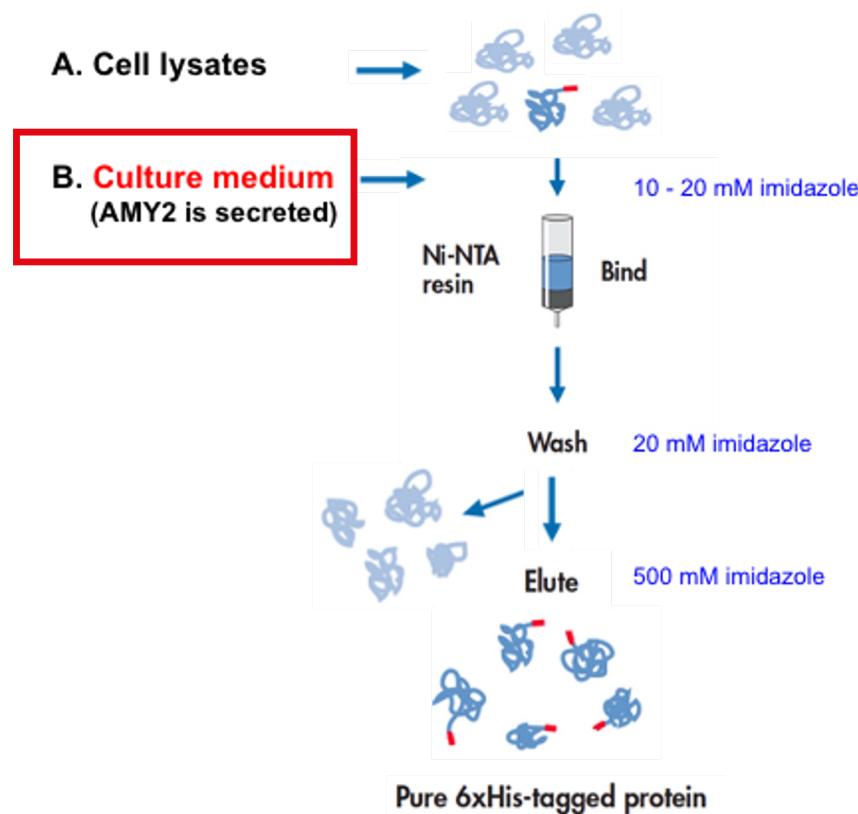


Imidazole

His-tagged proteins are eluted by competitive displacement with imidazole

Source: The QIAexpressionist Handbook,
Qiagen

Purification of AMY2-His from Cell Culture Medium



LABEL COLUMN FRACTIONS Group #

Total = TM+ (culture medium transfected cells)

FT = flow through

W = wash

E = elution

Adapted from the QIAexpressionist Handbook,
Qiagen